The Correlation of Somatic Characters and Chromatin Rod-Lengths, being a Further Study of Chromosome Dimensions.

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(With 5 Text-figures.)

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Introduction.

In a recent paper * dealing with chromosome dimensions in numerous organisms, I have shown that throughout the animal kingdom lengths of component rods appear to constitute members of a general series in arithmetical progression, whereas only three diameters exist, viz. ·21 μ in Protozoa, and ·42 and ·83 μ in low and higher Metazoa respectively.

Consideration of the results given has, moreover, led to the enunciation of an hypothesis which postulates a series of cycles in the course of phylogeny. It is suggested that the chromatin granules of the simplest Protozoa have been converted into rods by purely linear growth, accompanying evolutionary development and increasing somatic complexity, and, since the rate of this growth cannot have been the same in all chromosomes, rods of various lengths have been evolved ; examples of such complexes can be seen in *Ciliata* and other highly differentiated Protozoa. A stage in phylogeny was later reached when a maximum rod-length had been attained, such limit having been imposed by spindle mechanism or other physical conditions ; when this occurred chromatin units conjugated in fours, and the normal thread-width was thus doubled. The chromosomes, reduced in number, then segmented transversely into numerous spheres of the new diameter, and the process, which approximately re-established the number of chromosomes previously seen, enabled them to enter a fresh course of linear growth accompanying further evolutionary development. In this manner the complexes of low Metazoa may have evolved from those of Protozoan ancestors.

When the length-limit of chromosomes was again reached, conjugation of units once more occurred, and this was followed as before by segmentation into spheres of the new diameter ; the last named having been thus doubled, became identical with that now found in organisms belonging to phyla above and including Nemathelminthia. Thus the chromatin thread-width of the high Metazoa may have evolved from that of the lower.

This hypothesis seems to accord with phenomena, for I have been able to find in the animal kingdom examples that apparently represent stages of

* "A Metrical Analysis of Chromosome Complexes," Phil. Trans. Roy. Soc. ser. B, vol. 203, 1912.

transition to a greater thread-width: it is, however, impossible to prove this phylogenetic cycle with the meagre data at present available. If it is eventually established, we must realize that an attempt to correlate somatic characters and individual chromosomes must fail the moment that we consider any but the most closely allied organisms: at definite periods a complete rearrangement of units has occurred, and, since the subsequent rate of growth must have varied in different chromosomes under different conditions of environment, we have no reason for assuming correspondence between rods of the same length found in the germ-cells of widely separated organisms. Within the limits of a genus, however, it may be possible to trace somatic differences to differences in chromatin growth, for closely allied animals must have developed along the same or parallel lines, and we may therefore be able to identify corresponding chromosomes in their respective complexes. In the paper already referred to I have given camera lucida drawings of chromosome rods composing the complexes of several species of *Stenobothrus*, and have shown that the latter can be individually distinguished by the presence or absence of certain rod-lengths; I now propose to deal with another species of this genus in order to show that this phenomenon is probably common to all its members. Moreover, the comparative study of allied species may enable us to establish some correlation with respect to length of chromosomes and somatic characters.

Material and Methods.

Stenobothrus curtipennis, which belongs to the tribe Tryxalidæ and the family Acridiidæ, is not found in the British Isles, and I am indebted for the material to the kindness of Prof. H. S. Davis, who sent me testes fixed in Hermann's solution and embedded in paraffin, from the University of Florida, Gainesville, U.S.A. The sections were cut 8 μ thick and stained with Heidenhain's iron hæmatoxylin, the mordant used being an aqueous solution of iron alum. The preparations were studied by means of a Zeiss apochromatic oil-immersion objective of 2 mm. focus and N.A. 1·30, in conjunction with compensating oculars nos. 6, 12, and 18: I have used throughout the holoscopic oil-immersion substage condenser made by Messrs. Watson, of High Holborn, London.

All drawings were made with the aid of a large Abbe camera lucida at a magnification of three thousand and forty-eight diameters, the magnification being estimated by means of a stage-micrometer graduated to read one-hundredth of a millimetre. When necessary, resolution was facilitated by interposing a Gifford screen.

In order to avoid error due to foreshortening, drawings have been made only of chromosomes that lay at right angles to the microscopic line of vision

throughout their entire length, and errors in draughtsmanship have been minimised by drawing each individual chromosome many times; the measurements given should therefore represent the true dimensions with as great accuracy as can be obtained with the means now at our disposal.

Spermatogenesis.

The testes are two ovoid bodies lying in the middle of the abdomen dorsally to the alimentary canal; they are composed of tubular follicles tapering towards the ends and divided into numerous tracts and cysts. The primary and secondary spermatogonia lie at the extreme anterior end of each follicle, and next to these are large areas occupied by cells undergoing the growth-period; no resting stage occurs between the maturation mitoses, and the next portion of the follicle is accordingly occupied by both primary and secondary spermatocyte divisions. The posterior end contains spermatids undergoing transformation to unripe and ripe spermatozoa. As I have already pointed out in the case of *S. viridulus*, all cells in one cyst are not at precisely the same stage of development, and in a transverse section mitotic figures and resting stages can be seen lying side by side.

The primary spermatogonia are arranged in a cluster at the extreme anterior end of the follicle, whereas the secondary spermatogonia, more posteriorly placed, are in greater numbers and appear to be without definite arrangement in the cyst. The chromatin during the resting stages is disposed in granules upon linin threads, and the nucleus is apparently a complete reticulum. In the prophase of division this network breaks into numerous filaments, which shorten and condense until seventeen compact and smooth chromosomes are seen lying in the equatorial plane; these are divisible into eight pairs and one odd chromosome, which corresponds with the monosome of Davis and the heterotropic, accessory, and X chromosome of other writers.

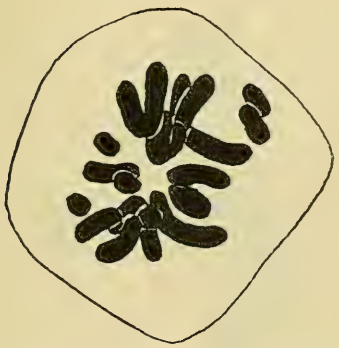


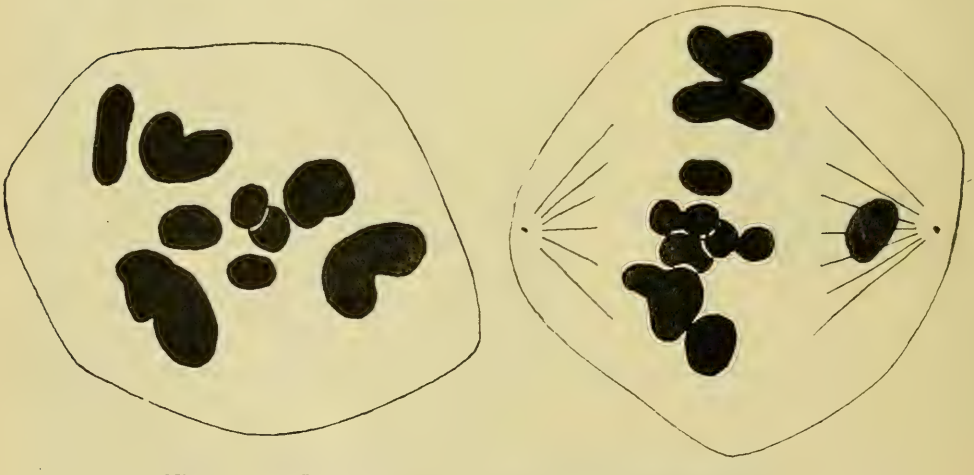
Fig. 1.—Spermatogonial metaphase.

The sixteen ordinary chromosomes are graded in length and individually composed of two equal rods, of which one passes to each pole in the subsequent anaphase: the plane of cleavage is invariably parallel to the major axes of these rods, which appear to be indivisible units. The diameter of the ordinary rods is constant, whereas that of the odd chromosome is greater and varies throughout its length; the latter is thus easily distinguishable from

the other members of the complex. Fig. 1 shows a polar view of this metaphase, and seventeen chromosomes are seen in the equatorial plane.

The last spermatogonial division is followed by a long period of growth, and large tracts of the follicle are seen occupied by cells undergoing this stage. The chromatin is again disposed in granules upon a network of linin threads, but the odd chromosome takes no part in the general dissociation and remains as a darkly staining and homogeneous body apposed to the nuclear membrane. The prophase of the first maturation division is characterized by the fission of individual granules and the breaking of the network into numerous double filaments ; these, which are at first long and ragged, conjugate in pairs and condense into the usual tetrads, appearing as rings, crosses, and figures of eight. The closeness with which the component rods are folded upon one another makes resolution extremely difficult, but size-relationships corresponding with those seen on the spermatogonial spindles are again recognizable, and each tetrad is undoubtedly composed of four equal rods in juxtaposition. Since these rods are similar to those of the earlier mitoses, the total amount of chromatin remains unchanged, and the

Fig. 2.—The Growth Period.



Figs. 3 & 4.—Polar and lateral views of first maturation mitosis.

eight tetrads are collectively equivalent to the sixteen ordinary spermatogonial chromosomes. I have failed to determine whether this division is reductional or equational, but this is immaterial, for either this or the next mitosis must separate paternal and maternal elements. Figs. 3 and 4, representing respectively polar and lateral views of this division, show the eight

tetrads and the odd chromosome, and in the latter the odd chromosome is seen passing entire to one pole, while the ordinary chromosomes are preparing for or actually undergoing fission in the equatorial plane.

Fig. 5.—Second maturation mitosis.

The second maturation division immediately follows the first, and the complex is composed of eight or nine chromosomes, the difference depending upon the odd chromosome, which is found in only 50 per cent. of these cells. As in the case of the spermatogonial metaphases, each ordinary chromosome is composed of two equal rods, and the same size-relationships are again apparent. Fig. 5 is an example of this metaphase seen from the polar aspect.

The transformation from spermatids to unripe and ripe spermatozoa is similar to that already described for these organisms by myself and other writers. The chromosomes become dissociated into minute granules, which at first stain only slightly with the iron hæmatoxylin ; the appearance of the "centrosome" is accompanied by elongation of nucleus and cytoplasm, the latter eventually constituting a long thread-like tail.

Dimensions of the Chromosomes.

The diameter of all component rods of the ordinary spermatogonial chromosomes is ·83 μ, and these consequently differ from one another only in length. The complex is divisible into two groups represented respectively by three long and five short pairs: this grouping accords with that of Davis, and with my own upon other members of the genus. Moreover, the lengths of the component rods of the five short pairs are respectively 1·7, 2·1, 2·5, 2·9, and 3·3 μ, and therefore constitute consecutive members of a series in arithmetical progression ; those of the three long pairs also belong to this series, but are alternate instead of consecutive, being respectively 5·0, 5·8, and 6·7 μ.

The tetrads or primary spermatocyte chromosomes cannot be measured accurately for their outlines are irregular, but a careful study of the filaments condensing during the preceding prophase leaves little doubt that they are individually composed of rods of the above dimensions.

The secondary spermatocyte complex is the most favourable for the measurement of chromosomes, since overlapping does not occur and individuals are composed of pairs of rods as in the spermatogonial mitoses. The diameter of the component rods of ordinary chromosomes is again ·83 μ, and the lengths are respectively 1·7, 2·1, 2·5, 2·9, 3·3, 5·0, 5·8, and 6·7 μ, *i. e.*, identical with those of the spermatogonia. The odd chromosome, found

in 50 per cent. of these cells, is again easily recognizable on account of its great breadth. I have already produced evidence to show that rods composing ordinary chromosomes of organisms above and including Nemathelminthia have a constant diameter, viz. ·83 μ, and that their lengths constitute members of a general series in arithmetical progression ; and the chromosome measurements of *S. curtipennis* therefore afford further support to this assumption.

The accompanying figure (fig. 6) shows the complexes of *Stenobothrus parallelus*, *S. viridulus*, *S. bicolor*, and *S. curtipennis*, the four complexes being respectively marked A, B, C, and D. In my recent paper I have identified rod-lengths of the general series by numerals, which are again used and are placed above the corresponding chromosomes. The drawings show component rods, each spermatogonial and secondary spermatocyte chromosome being composed of two and each primary spermatocyte chromosome of four.

The rod-lengths of the five short chromosomes appear to be the same in all four species, whereas those of the three long chromosomes are not identical in any two : in *S. parallelus* they correspond respectively with Chromosomes 14, 15, and 17 of the general series, in *S. viridulus* with Chromosomes 13, 15, and 17 ; in *S. bicolor* with Chromosomes 11, 13, and 17, and in *S. curtipennis* with Chromosomes 11, 13, and 15.

If now we assume that the chromatin is directly concerned with the transmission of the hereditary characters—and we have many reasons for assuming this—we must look for the cause of somatic differences between these species in the three long chromosomes, for the respective nuclei appear to differ only in the lengths of these. Moreover, we must try to discover how these differences in rod-lengths have occurred, for the problem of chromosome function must be intimately connected with such differences.

Let us firstly assume that chromosome rods throughout the animal kingdom are of fixed lengths, and that morphological similarity is invariably accompanied by functional similarity. This assumption carries with it the further assumption that in the course of evolution certain rods have disappeared from each complex, local conditions having determined which should persist and which should be eliminated : moreover, it postulates a greater number of chromosomes in primitive than in highly organized types, and we must expect to find allied organisms possessing many chromosome lengths in common. The former of the last-named corollaries is, however, not supported by actual investigations, and with regard to the latter I have already shown that *Forficula* does not possess one rod-length in common with *Stenobothrus*. We are accordingly faced by a complete contradiction, for, if a definite chromosome rod-length is invariably correlated with a definite set of somatic characters, no such set of characters can be possessed by both earwings and grasshoppers—members of sister families.

Fig. 6.

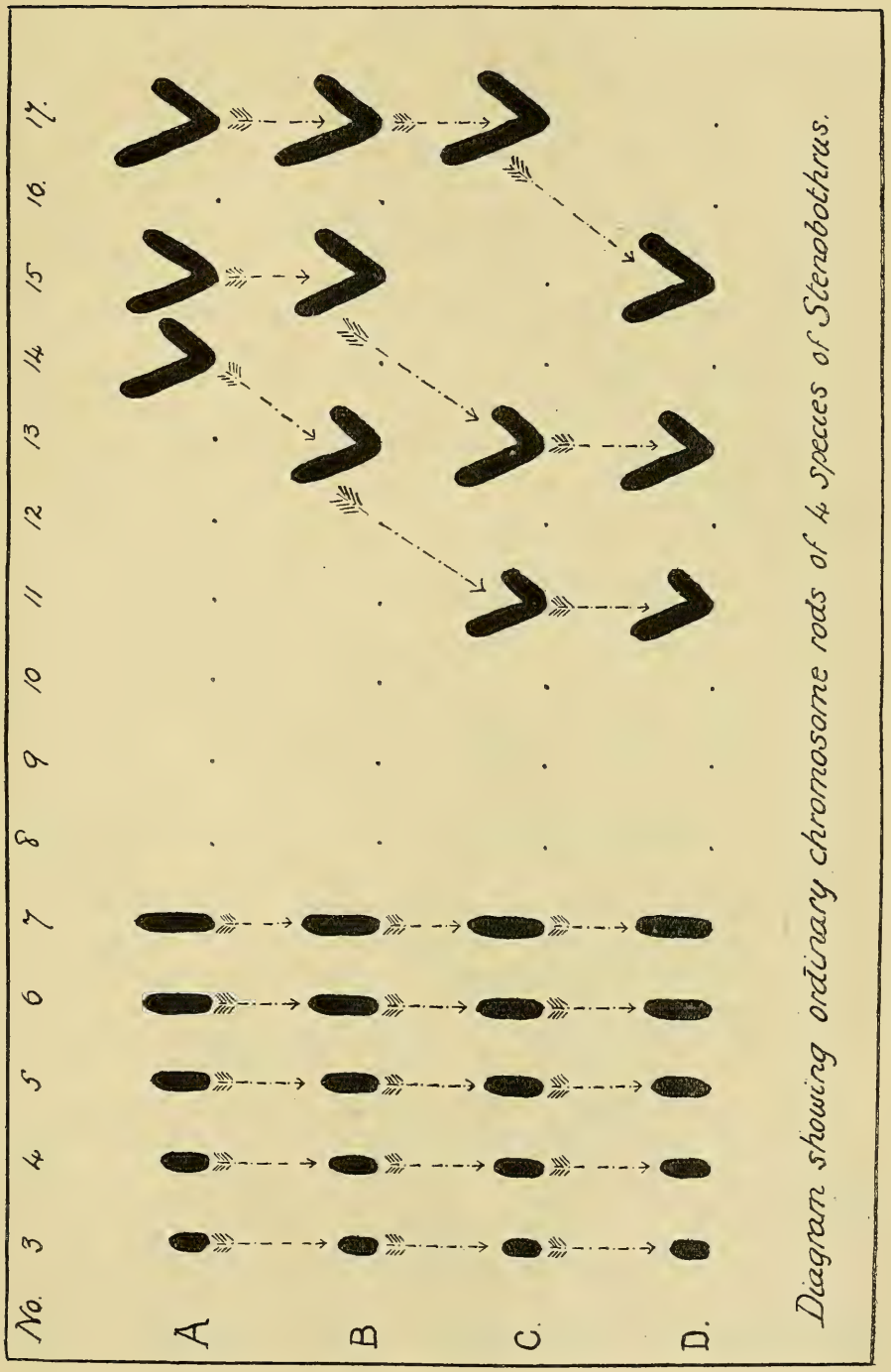


Diagram showing ordinary chromosome rods of 4 species of Stenobothrus.

A. *Stenobothrus parallelus.* B. *S. viridulus.* C. *S. bicolor.* D. *S. cuvipennis.*

The second and alternative hypothesis postulates a continuous linear growth of chromosomes in the course of phylogeny, and is based upon data that support direct correspondence between the degree of somatic complexity of an organism and the total volume of its germ-cell chromatin; it moreover offers a logical explanation of the evolution of various rod-lengths. The measurements that I have given can be only approximations, and the difference between terms of the general series is probably smaller than that shown, but these measurements suffice to prove differences between complexes of allied species; and it is only reasonable to suppose that such differences are of comparatively recent origin and have evolved by some continuous process accompanying the somatic differentiation of the species. In certain cases the process may have been complicated by degeneration, possibly resulting in the complete disappearance of a particular chromosome from the complex, but, even if this additional factor is eventually established, I am aware of no reasons for discarding the assumption that the guiding principle in complex formation is and has been a purely linear growth of component rods.

I have already pointed out that if this second hypothesis is subsequently proved, morphological identity of chromosomes can be no guide to functional identity outside the narrowest limits of our classification; but in the case of allied species, which must have evolved along almost parallel lines, we may reasonably hope to establish correlation of individual chromosomes of the respective complexes, and thus form a basis upon which to attempt correlation of rod lengths and definite somatic characters.

Let us therefore consider again the complexes of these four species of *Stenobothrus*. The lengths of the five short chromosomes are the same in all cases, and their identities appear consequently to be established; the three long chromosomes, however, are not the same in any two complexes, and correspondence is therefore not at once apparent. If we accept the first hypothesis, which postulates invariable correspondence between definite rod-lengths and definite sets of somatic characters, we must realize that no long chromosome is common to all four species; Chromosome 11 is absent in *S. parallelus* and *S. viridulus*, Chromosome 13 is absent in *S. parallelus*, Chromosome 14 is absent in *S. viridulus*, *S. bicolor*, and *S. curtipennis*, Chromosome 15 is absent in *S. bicolor*, and Chromosome 17 is absent in *S. curtipennis*. If, on the other hand, we accept the second hypothesis, which postulates continuous linear growth of rods, we must realize that in these complexes the short, medium, and long chromosomes of the three are probably respectively identical. Thus Chromosome 13 of *S. viridulus* does not correspond with Chromosome 13 of *S. bicolor* and *S. curtipennis*, but corresponds with Chromosome 11 of the two latter and with Chromosome 14 of *S. parallelus*, for these chromosomes constitute the shortest member of the long group in each case; similarly, Chromosome 17 of *S. parallelus*,

S. viridulus, and *S. bicolor* is not unrepresented in *S. curtipennis*, but is functionally identical with Chromosome 15, which has not yet grown sufficiently in the last-named to be classed in the higher category. Correspondence between the remaining long chromosomes can be seen in the diagram by following the arrows, which have been inserted to show identities in all cases.

If the difference between terms of the general series is eventually found to be smaller than half the rod diameter, chromosomes that we now class as consecutive must be separated by intermediate lengths, and those that we class together may, as a result of more accurate means of measurement, be shown to have minutely differing lengths: this, however, cannot affect the three long chromosomes of *Stenobothrus*, for their respective lengths are such that mistake in identification is impossible. We must nevertheless remember that the correspondence indicated by the arrows is based upon a pure hypothesis, and that at present we possess no direct evidence in support of this correspondence.

Turning now to the consideration of somatic characters, we find that these grasshoppers are individually distinguished by slight but clearly defined differences. The following descriptions give the principal characteristics and are quoted from the works of Bolivar, Burr, and Kirby.

STENOBOTHRUS PARALLELUS.

1. Very variable in colour.
2. Antennæ longer in male than in female.
3. Pronotum with transverse furrow nearer to posterior than to anterior border.
4. Lateral carinæ of pronotum almost parallel or slightly approximating about the middle.
5. Elytra do not reach end of abdomen in male; in female do not reach beyond fourth abdominal segment.
6. Mediastinal area of elytra extended abruptly towards the apex and extended round base, forming a rounded lobule; anterior margin of elytra convex round base.
7. Wings rudimentary.
8. Length of body, male 14–15 mm., female 18–21 mm.

STENOBOTHRUS VIRIDULUS.

1. Green varied with darker.
2. Antennæ longer in male than in female.
3. Pronotum with transverse furrow midway between anterior and posterior borders.
4. Lateral carinæ of pronotum slightly angled near anterior border, rounded slightly posteriorly.

5. Elytra fully developed in both sexes.
6. Mediastinal area of elytra gradually extended towards the apex, prolonged to length of anterior border and not lobulate at base. Anterior border straight.
7. Wings developed.
8. Length of body, male 13–15 mm., female 20–24 mm.

Stenobothrus bicolor.

1. Very variable in colour.
2. Antennæ of equal length in both sexes.
3. Pronotum with transverse furrow nearer to anterior than to posterior border.
4. Lateral carinæ of pronotum sharply angled in anterior part, diverging towards anterior and posterior borders.
5. Elytra fully developed in both sexes.
6. Mediastinal area of elytra extended abruptly towards the apex and extended round base, forming a rounded lobule ; anterior margin of elytra convex round base.
7. Wings developed.
8. Length of body, male 15–16 mm., female 19–24 mm.

Stenobothrus curtipennis.

1. Variable in colour.
2. Antennæ longer in male than in female.
3. Pronotum with transverse furrow nearly in middle.
4. Lateral carinæ of pronotum straight and parallel.
5. Elytra very short.
6. Mediastinal area of elytra extended abruptly towards the apex and extended round base, forming a rounded lobule ; anterior margin of elytra convex round base.
7. Wings very short in female ; in male equal in length to body.
8. Length of body, male 17·5 mm., female 17·5 mm.

Let us now try to correlate these characters and the chromosome rod-lengths of the respective complexes. We will firstly consider the lateral carinæ of the pronotum ; these are parallel or slightly approximating in *S. parallelus*, *S. viridulus*, and *S. curtipennis*, but sharply angled in *S. bicolor*. This characteristic is distinctive, for Burr has pointed out that by it alone we can distinguish the last-named from the other three species. Now if correlation is evident, we must expect to find corresponding chromosomes of the same length in *S. parallelus*, *S. viridulus*, and *S. curtipennis*, but of a different length in *S. bicolor* ; if, however, we follow the arrows in the diagram we see that no long chromosome fulfils these conditions, and correlation is therefore not established.

Taking as a second example the mediastinal area of the elytra, we must expect to find corresponding chromosomes of the same length in *S. parallelus*, *S. bicolor*, and *S. curtipennis* and of a different length in *S. viridulus*, for in the three first named the area extends abruptly towards the apex whereas in the last it extends gradually. We again fail to observe such a chromosome ; and the same absence of correlation is noticeable with respect to the other characters on the list with the exception of colour, which is variable and untrustworthy.

Moreover, we are not more successful if we assume the first hypothesis, which postulates unchanging rod-lengths. Disregarding the arrows in the diagram and considering correspondence to depend entirely upon length, we find that the length of antennæ and angle of the lateral carinæ may be correlated with Chromosome 15 in *S. parallelus*, *S. viridulus*, and *S. curtipennis* ; but the fact that no other characteristics appear to correspond with chromosome lengths makes justification for this assumption doubtful. Furthermore, the genus *Stenobothrus* has been divided by Bolivar and other systematists into subgenera, and these species are now classified as follows :— *Chorthippus parallelus*, *Chorthippus curtipennis*, *Ornocestus viridulus*, and *Stauroderus bicolor* : it is noteworthy that the two whose complexes show the greatest differences in rod-lengths should thus be classed together.

Four explanations may be put forward to account for this failure. Firstly, we may assume that my measurements are inaccurate ; this, however, seems unlikely, for great care has been exercised, and the lengths of the long chromosomes are such that relative error should be impossible. Secondly, we may assume that the lengths of the five short chromosomes are not respectively identical in all the species ; in this case the characters mentioned may be correlated with these and not with the three long chromosomes : if, however, the principal somatic differences, upon which systematists have based their classification, are not traceable to obvious differences in long chromosomes, why should they be traceable to imperceptible differences in short chromosomes, and, if they are so traceable, to what are the obvious differences in the former due ? Thirdly, the arrows may be misleading : Chromosome 17 of *S. parallelus*, *S. viridulus*, and *S. bicolor* may, for example, correspond in *S. curtipennis* with Chromosome 13 and not 15, in which case the last named chromosome corresponds with the medium instead of the longest member of the three, and is accordingly functionally identical with Chromosome 15 of *S. parallelus* and *S. viridulus* and Chromosome 13 of *S. bicolor*. This is undoubtedly possible, if rods are continuously increasing in length, for a long chromosome may in the course of evolution be overtaken and passed by one that was shorter, and the latter may consequently be mistaken for the former : if this occurs, we must realize that measurements cannot always be a trustworthy index to functional correspondence even in the most closely allied organisms. Lastly, if the

chromosomes of a complex are qualitatively different, as we have reasons for believing, each must be concerned with a definite set of characters : difference in length of two corresponding chromosomes may therefore be connected with differences in several characters, and, even if the character under consideration is included in these, other factors are equally bound up in the chromatin rod, and may be responsible for apparently irreconcilable lengths.

In the circumstances I am inclined to think that the fourth explanation, possibly coupled with the third, will eventually be found to account for our present failure. It is difficult to believe that the obvious somatic differences mentioned in our list are not in some way connected with the lengths of the three long chromosomes, but until a thorough analysis has been made of both internal and external characteristics of these species we cannot hope to correlate somatic characters and chromosome rod-lengths in the genus. In 1908 McClung indicated a course of investigation upon Acridiidæ to be carried out upon these lines, but I have seen no paper by him on the subject : possibly he and his followers have been able to throw some light upon this difficult problem.

Resumé.

Each ordinary spermatogonial and secondary spermatocyte chromosome of *S. curtipennis* is composed of two equal rods, and each primary spermotocyte chromosome of four. The diameter of these rods is invariably ·83 μ and consequently lends further support to the assumption that the chromatin thread-width is constant in all organisms above and including Nemathelminthia.

The lengths of the ordinary rods constitute members of a general series in arithmetical progression ; the five short chromosomes are respectively identical with those of other members of the genus, but the lengths of the three long chromosomes once more enable the species to be identified.

A comparison between *S. parallelus*, *S. viridulus*, *S. bicolor*, and *S. curtipennis* fails to establish correlation of somatic characters and chromosome rod-lengths, but we have reason for believing that the obvious characteristics upon which identification is based are in some way connected with the three long chromosomes : our present failure is probably due to ignorance of the less obvious somatic characteristics and to the lack of trustworthy methods of identifying corresponding chromosomes in the respective complexes.

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